PHARMACY: Jurnal Farmasi Indonesia p-ISSN 1693-3591
(*Pharmaceutical Journal of Indonesia*) e-ISSN 2579-910X
Vol.18 No. 02 Desember 2021: 476-484

The Effect of Black Cumin Seed Oil on Active Smokers Based on Interleukin-1β Activity and Neutrophil

Shabrina Arwi Laily[1,2], Titiek Hidayati[3], Akrom[1,2,4,5]*, Vivi Sofia[1,2,4]

[1]Department of Pharmacy, Universitas Ahmad Dahlan, Indonesia
[2]Magister Pharmacy Program, Pharmacy Faculty, Universitas Ahmad Dahlan, Indonesia
[3]Health and Family Medicine, Medical and Health Science Faculty,
Universitas Muhammadiyah Yogyakarta, Indonesia
[4]Pharmacology and Clinical Pharmacy Department, Pharmacy of Faculty,
Universitas Ahmad Dahlan, Indonesia
[5]Ahmad Dahlan Drug Information and Crisis Center, Indonesia

*Corresponding author email: akrom@pharm.uad.ac.id

Received 30-07-2021 **Accepted 0**7-08-2021 **Available online** 31-12-2021

ABSTRACT

Health problems due to exposure to cigarettes cause the body to fight against the occurrence of immune responses, one of which is leukocytes. The most common types of leukocytes are neutrophils, which are about 50-70% with a function as the body's line of defense against foreign substances. One of the toxic components in cigarettes is nicotine. Nicotine in cigarettes can induce the production of IL-1β. Black Cumin Seed Oil (BCSO) in various studies has been widely used as a supplement, especially as an immunomodulator. This study used a single-blind Randomized Controlled Trial (RCT) method, with a total of 39 test subjects divided into four groups for 30 days. Group 1 received placebo 3x1 capsules/day, group 2 received BCSO 3x1 capsules/day, group 3 received BCSO 3x2 capsules/day, and group 4 received BCSO 3x3 capsules/day. Data analysis used a one-way ANOVA test to see the average neutrophils between treatment groups. While the average levels of IL-1β using the method of *Kruskall Wallis.* The test results are said to be significant if p 0.05. The p value of the ANOVA test was 0.16 neutrophil levels. In *Kruskal Wallis,* the –p value for IL-1β is 0.43. BCSO administration does not affect IL-1β levels in smokers and BCSO doses 3x1 capsules/day can reduce neutrophil levels.

Keywords: BCSO, neutrophils, randomized controlled trial, smokers.

Introduction

The impact of smoking is one of the biggest health problems in the world. The World Health Organization (WHO) states that smoking can cause health problems that are the cause of death of approximately 6 million people per year. The risk of death from smoking in active smokers is higher than passive smokers (Hidayati et al., 2019). Cigarette

smoke is high in free radicals and triggers a decrease in antioxidant capacity. The increase in free radicals in smokers and a decrease in the body's immune system will trigger various systemic diseases such as rheumatoid arthritis, cancer, diabetes mellitus, cardiovascular disease, neurodegenerative and asthma (Suryadinata et al., 2017). The three main toxic components found in cigarettes are carbon monoxide, tar and nicotine (Janah & Martini, 2017). High levels of tar and nicotine in the body cause immunological changes by suppressing the phagocytic activity of neutrophils (Yamaguchi, 2019).

Neutrophils are a type of leukocyte that plays a role in the body's defense mechanism (Utami et al., 2013). Neutrophils play a role in the immune response due to smoking by increasing free radicals in the body. Exposure to tobacco smoke can also cause oxidative stress and trigger an inflammatory response in the lungs (Suryadinata et al., 2017). During inflammation, neutrophils in the blood can increase four times to five times the normal number (Nusa et al., 2016).

Interleukin 1 beta (IL-1β) plays an important role in inflammation and immunity. IL-1β is produced by monocytes, macrophages, neutrophils and also by other cell types such as fibroblasts and epithelial cells (Moeintaghavi et al., 2017). Exposure to cigarette smoke causes changes in the epithelial structure which results in the release of proinflammatory cytokines such as TNFα and IL-1β by alveolar macrophages (Wahyuni, 2017).

Increased levels of cytokines and/or chemokines released by inflammatory cells after exposure to cigarette smoke function as *chemoattractants* that will exacerbate inflammation (Zuo et al., 2014).

Traditional medicine is widely used in improving the health of Indonesian people. Traditional medicine refers to efforts to strengthen natural healing. One of the traditional medicinal plants that is starting to get attention for its benefits is black cumin (Marlinda, 2015). Black cumin seeds are believed to maintain human health and are used to treat various diseases such as hypertension, rheumatism and treat various bacterial infections. In addition, it is important to maintain kidney, bile, liver function, and is used to improve the immune system (Sulistiawati, 2014). Black cumin is useful as an immunomodulator by increasing antibody titers and increasing monocytes and decreasing neutrophils (Amanulloh et al., 2019). The important ingredients of black cumin are thymoquinone, dithymoquinone, thymohydroquinone and thymol which can activate and awaken the immune system with its ability to increase levels of helper T cells, suppressor T cells and natural killer cells to maintain homeostasis (Ningtyas, 2012). To date, the benefits of taking BCSO on neutrophil counts and IL-1β levels have not been studied. Based on this description, this study was conducted to

determine the effect of giving BCSO for 30 days with various doses on the levels of neutrophils and IL-1β in healthy volunteers who are active smokers.

Research Method

Materials

Tools and materials used in this study included primary and secondary data collection sheets using a case report form (CRF), blood collection equipment, and a set of tools for blood analysis. The CRF was used to collect demographic and lifestyle data. Neutrophil levels were analyzed using a Hematology Analyzer. Measurement of vital signs, filling out CRF, and nutritional status and blood collection were carried out at residents' homes. Complete blood count was carried out at Nur Hidayah Hospital.

Method

This research is an experimental study with a Randomized Controlled Trial (RCT) design. The sampling technique used is purposive sampling, where the researcher determines the sample based on predetermined criteria. Eligible subjects were divided into four groups by simple random sampling. Subjects received either three-dose BCSO capsules or a placebo 3x1 for 30 days. Reviewing health conditions and side effects is carried out daily through smartphones. Every three days, researchers made visits to test subjects to ensure compliance with BCSO administration. The compliance of the test subjects was measured by calculating the previously given BCSO difference.

Data Analysis

Analysis of the average levels of neutrophils used a one-way ANOVA test which previously had met the normality and homogeneity tests to see whether there was a significant difference between the treatment groups. The analysis compares the average levels of IL-1β between treatment groups using Kruskal Wallis

Results and Discussion

General Description of Respondents

The subjects of this study consisted of 39 active smokers volunteers who were divided into 4 treatment groups located in the working area of the Jetis 1 Public Health Center in Bantul. Demographic conditions of research subjects according to gender, education, status, occupation, smoking status, age, length of smoking (years) and number of cigarette consumption (sticks/day).

As shown in Table 1. the characteristics of respondents as subjects. The subject is male and smokers (100%), this is in accordance with research by Chinwong et al. (2018) where smoking behavior is higher among men than women and the average number of daily cigarette consumption is higher in men than women. The level of education is mostly senior high school (SHS) (61.5%). Level of education affects smoking behavior. Education can affect a person's way of thinking and behavior

related to the risks that arise for health due to smoking (Juliansyah & Rizal, 2018). Most of them are married (66.7%) and their work is dominated as laborers (46.2%). The mean age was 40 years, the average length of smoking was 18 years and the average number of cigarettes consumed was 10 cigarettes/day. Examination of vital signs, namely the average blood pressure showed a result of 139/87 mmHg. There is a relationship between increased blood pressure and smoking behavior. Factors related to increased blood pressure include the number of cigarettes smoked, how to smoke and the frequency of smoking duration (Tisa, 2012).

Based on the Table 1, analysis was carried out *One Way Anova*, where the p-value in the age group was 0.80, blood pressure was 0.52, length of smoking was 0.63 and the number of cigarettes consumed per day was 0.66. From the results of the overall analysis, it was found that -p value > 0.05, which means there was no significant difference between in the placebo group, BCSO 3x1 capsules/day, BCSO 3x2 capsules/day and BCSO 3x3 capsules/day. This is possible because the length of exposure to cigarette smoke experienced by each individual is different.

Analysis of IL-1β Levels Between Treatment Groups

Nicotine in cigarettes can induce the production of IL-1β significantly in in vitro studies (Wu et al., 2014). This study is expected to show that BCSO can reduce levels of IL-1β. As shown in table 2, the average IL-1 after administration of BCSO in the placebo group was 16.61 pg/mL, the BCSO 3x1 group was 24.67 pg/mL, the BCSO 3x2 group was 20.83 pg/mL and the 3x3 group was 17.61 pg/mL. In normal healthy adults, serum IL-1β concentrations range from 0.5 to 12 pg/mL (O'Neill et al., 2013). When compared to normal values between IL-1β and placebo, it was not in the normal range, so BCSO administration did not affect IL-1β levels. The difference in IL-1β expression which is higher than in the placebo group is a sign of a more active inflammatory process (Astawa et al., 2017). Research by (Zikriah, 2014) stated that a dose of 250mg/kg black cumin ethanol extract was able to suppress lipopolysaccharide-induced interleukin 1β levels. Another study stated that black cumin extract was able to inhibit IL-1β (Amartey et al., 2019).

The average level of IL-1β levels obtained was analyzed by the Kruskal Wallis test. As shown in table 2, the p-value is 0.43 > 0.05, which means that there is no significant difference between the average level of IL-1β in the placebo group, BCSO 3x1 capsules/day, BCSO 3x2 capsules/day and BCSO 3x3 capsules/day.

Analysis of the Level of Neutrophils between Treatment Groups

Neutrophils were the most common type of leukocyte, about 50-70% among other leukocyte cells.

PHARMACY: Jurnal Farmasi Indonesia p-ISSN 1693-3591
(*Pharmaceutical Journal of Indonesia*) e-ISSN 2579-910X
Vol.18 No. 02 Desember 2021: 476-484

Table 1. Demographic characteristics of subjects at Jetis 1 Health Center Bantul

Respondents' characteristics		Treatment				Percentage (%)		Sig (2-tailed)
		Placebo 3x1 capsules /day	BCSO 3x1 capsules /day	BCSO 3x2 capsules /day	BCSO 3x3 capsules /day	Each Treatment	Total	
Type Gender	Male	9 (23.1%)	11 (28.2%)	10 (25.6%)	9 (23.1%)		100%	
	Female							
Education	Elementary		3 (50%)	1 (16.7%)	2 (33.3%)	6 (100%)	15.4 %	
	JHS	2 (40%)	2 (40%)	0	1 (20%)	5 (100%)	12.8 %	
	SHS	7 (29.2%)	5 (20.8%)	7 (29.2%)	5 (20.8%)	24 (100%)	61.5 %	
	S1/S2/S3	0	1 (25%)	2 (50%)	1 (25%)	4 (100%)	10.3%	
Marital status	Married	8 (30.8%)	5 (19.2%)	6 (23.1%)	7 (26.9%)	26 (100%)	66.7%	
	Unmarried	1 (7.7%)	6 (46.2%)	4 (30.8%)	2 (15.4%)	13 (100%)	33.3%	
Job	Private	4 (23.5%)	6 (35.3%)	5 (29.4%)	2 (11.8%)	17 (100%)	43.6%	
	Farmers	0	0	0	1 (100%)	1 (100%)	2.6 %	
	Laborers	5 (27.8%)	5 (27.8%)	3 (16.7%)	5 (27.8%)	18 (100%)	46.2%	
	Student	0	0	0	1 (100%)	1 (100%)	2.6 %	
	Retirees	0	0	1 (100%)	0	1 (100%)	2.6 %	
	Teachers	0	0	1 (100%)	0	1 (100%)	2.6 %	
Age		39.44±6.75	41.55 ± 12.32	36.4±15.23	41.11±14.63	39.64±12.41		0.80
Blood Plessure (mmHg)		141/92±23/16	138/87±18/9	143/91±30/15	135/80±9/7	139/87±21/13		0.52
Length of smoking (years)		15.89±8.84	20.64±13.39	14.8±11.97	20.56±15.33	18.03±12.43		0.63
Total consumption of cigarettes (cigarettes/day)		9.89±10.45	10.55±5.26	7.6±4.25	11.56±7.71	9.87±7.02		0.66

Bivariate analysis using one-way ANOVA, if $p<0.05$ there is a significant difference

Table 2. Average IL-1 levels after administration of placebo and BCSO for 30 days

Treatment group	N	Average IL-1β (pg/mL)	Sig – (2-tailed)
Placebo 3x1 capsules /day (control)	9	16.61	0.43
BCSO 3x1 capsules/day	9	24.67	
BCSO 3x2 capsules/day	12	20.83	
BCSO 3x3 capsules/day	9	17.61	
Total	39		

Bivariate analysis using Kruskal Wallis, if p<0.05 there is a significant difference

Neutrophils function as the body's line of defense against foreign substances, especially bacteria that are phagocytic and can enter infected tissues (Kiswari, 2014). According to previous research, smoking can increase neutrophil levels in the blood (Prihandari, 2016).

The immune system of smokers decreases in fighting bacterial infections compared to non-smokers so that it will increase the number of neutrophils (Zuo et al., 2014). In this study, it is hoped that the administration of BCSO can reduce neutrophil levels. As shown in Table 3, the mean neutrophils after being given BCSO for 30 days in the placebo group was 54, the BCSO 3x1 group was 52.87, the BCSO 3x2 group was 56.82 and the BCSO 3x3 group was 61.87. Table 2 shows the BCSO dose of 3x1 capsules/day can reduce neutrophil levels when compared to the placebo group. However, for BCSO 3x2 capsules/day and BCSO 3x3 capsules/day, there was no decrease in neutrophil levels. The difference in the results of the study could occur because the respondents did not comply when taking BCSO capsules. So that when compared between treatment groups, data are obtained that are less valid. Another study stated that the ethanolic extract of black cumin can be used as an anti-inflammatory agent by preventing the reaction of mast cells and decreasing neutrophil levels (Boskabady et al., 2010).

Table 3. Average neutrophil levels after giving placebo and BCSO for 30 days

Treatment Group	N	Average neutrophil	Standard deviation	Sig – (2-tailed)
Placebo 3x1 capsules/day (control)	9	54	7.55	0.16
BCSO 3x1 capsules /day	9	52.87	10.31	
BCSO 3x2 capsules /day	12	56.82	8.12	
BCSO 3x3 capsules /day	9	61.87	9.92	
Total	39			

Bivariate analysis using one-way ANOVA, if p<0.05 there is a significant difference

Analysis of the average level of neutrophil levels using one-way ANOVA test. Based on the results of the one-way ANOVA test in the table, the p-value is 0.16 > 0.05, which means that there is no significant difference between the mean neutrophil levels in the placebo group, BCSO 3x1 capsules/day, BCSO 3x2 capsules/day and BCSO 3x3 capsules/day.

Conclusion

The number of BCSO capsules consumed every day affects respondent compliance in consuming BCSO. The administration of BCSO did not affect IL-1β levels in smokers and the administration of BCSO 3x1 capsules/day could reduce neutrophil levels.

Acknowledgments

Thanks to the Ministry of Higher Education Research and Technology for providing research funding through research grants under the Higher Education Applied Research scheme and for all the volunteers who have participated in this research. Thank you to the Bantul Health Office for granting research permission and the Head of Jetis 1 PHC who has provided material and non-material supports.

Reference

Amanulloh, M., & Krisdayanti, E. (2019). Jintan Hitam sebagai Imunomodulator dan Anti Inflamasi pada Pasien Asma. *Jurnal Penelitian Perawat Profesional*, *1*(1). https://doi.org/10.37287/jppp.v1i1.32

Amartey, J., Gapper, S., Hussein, N., Morris, K., & Withycombe, C. E. (2019). Nigella sativa extract and thymoquinone regulate inflammatory cytokine and TET-2 expression in endothelial cells. *Artery Research*. https://doi.org/10.2991/artres.k.191114.002

Astawa, P., Bakta, M., Budha, K. (2017). Makrofag Pengekspresi Il-1β Serta Respons Inflamasi Sistemik Pada Fiksasi Interna Dini Fraktur Femur Tertutup Lebih Rendah Dibandingkan Dengan Yang Tertunda. *Indonesian Journal Of Biomedical Sciences*, *1*(3).

Boskabady, M. H., Mohsenpoor, N., & Takaloo, L. (2010). Antiasthmatic effect of Nigella sativa in airways of asthmatic patients. *Phytomedicine*. https://doi.org/10.1016/j.phymed.2010.01.002

Chinwong, D., Mookmanee, N., Chongpornchai, J., & Chinwong, S. (2018). A Comparison of Gender Differences in Smoking Behaviors, Intention to Quit, and Nicotine Dependence among Thai University Students. *Journal of Addiction*. https://doi.org/10.1155/2018/8081670

Hidayati, T., Akrom, A., Indrayanti, I., & Sagiran, S. (2019). Black cumin seed oil increase leucocyte and CD4Thelper number in sprague-dawley rats induced with dimethylbenzanthracene. *International Journal of Public*

Health Science (IJPHS). https://doi.org/10.11591/ijphs.v8i2.17930

Janah, M., & Martini, S. (2017). Hubungan Antara Paparan Asap Rokok Dengan Kejadian Prehipertensi Relationship Between Secondhand Smoke And Prehypertension. *Jurnal Manajemen Kesehatan Yayasan RS.Dr. Soetomo*, *3*(2). https://doi.org/10.29241/jmk.v3i1.75

Juliansyah, E., & Rizal, A. (2018). Faktor umur, pendidikan, dan pengetahuan dengan perilaku merokok di wilayah kerja Puskesmas Sungai Durian, Kabupaten Sintang. *Visikes: Jurnal Kesehatan Masyarakat*.

Kiswari, R. (2014). *Hematologi & Transfusi*.

Marlinda, L. (2015). Effectivityof Black Cumin Seeds Extract To Increase Phagocytosis. *Journal of Majority*.

Moeintaghavi, A., Arab, H. R., Rahim Rezaee, S. A., Naderi, H., Shiezadeh, F., Sadeghi, S., & Anvari, N. (2017). The Effects of Smoking on Expression of IL-12 and IL-1β in Gingival Tissues of Patients with Chronic Periodontitis. *The Open Dentistry Journal*. https://doi.org/10.2174/1874210601711010595

Ningtyas. (2012). Aktivasi Pemakaian Jinten Hitam (*Nigella sativa*) terhadap Respons Imun pada Gigi yang Mengalami Inflamasi. *Stomatognatic (J.K.G Unej)*, *9*(1).

Nusa, G., & Widyastiti, N. (2016). Perbedaan Neutrophil-Lymphocyte Ratio pada Subjek Bukan Perokok, Perokok Ringan dan Perokok Sedang-Berat. *Jurnal Kedokteran Diponegoro*, *5*(4).

O'Neill, C. M., Lu, C., Corbin, K. L., Sharma, P. R., Dula, S. B., Carter, J. D., Ramadan, J. W., Xin, W., Lee, J. K., & Nunemaker, C. S. (2013). Circulating levels of IL-1B+IL-6 cause ER stress and dysfunction in islets from prediabetic male mice. *Endocrinology*, *154*(9). https://doi.org/10.1210/en.2012-2138

Sulistiawati, Farida, R. M. (2014). Potensi Pemanfaatan Nigella sativa L. sebagai Imunomodulator dan Antiinflamasi. *Pharmaceutical Sciences and Research*, *1*(2), 65–77. https://doi.org/10.7454/psr.v1i2.3493

Suryadinata, R. V, Lorensia, A., & Sari, R. K. (2017). Perbedaan Asupan Nutrisi Makanan dan Indeks Massa Tubuh antara Perokok Aktif dan Non-perokok pada Usia Dewasa. *Indonesian Journal of Clinical Pharmacy*, *6*(3).

Tisa K, A. N. (2012). Hubungan Antara Kebiasaan Merokok Dengan Tekanan Darah Meningkat Karyawan Laki-Laki Di Nasmoco Semarang the Relation Between Smoking Habit With Increased Blood Pressure Male Employee in Nasmoco Semarang. *Jurnal Kesehatan Masyarakat Universitas Diponegoro*, *1*(2).

Utami, D. T., Prayitno, S. B., Hastuti, S., & Santika, A. (2013). Gambaran Parameter Hematologis pada Ikan Nila (Oreochromis Niloticus) yang diberi Vaksin DNA *Streptococcus iniae* dengan Dosis yang Berbeda. *Journal of Aquaculture Management and Technology*, *2*(4), 7–20.

Wahyuni, R. D. (2017). Aspek Imunologi Chronic Obstructive Pulmonary Diseases (Copd). *Medika Tadulako: Jurnal Ilmiah Kedokteran Fakultas Kedokteran Dan Ilmu Kesehatan*, *4*(1).

Wu, L., Zhou, Y., Zhou, Z., Liu, Y., Bai, Y., Xing, X., & Wang, X. (2014). Nicotine induces the production of IL-1β and IL-8 via the α7 nAChR/NF-κB pathway in human periodontal ligament cells: An in vitro study. *Cellular Physiology and Biochemistry*, *34*(2). https://doi.org/10.1159/000363011

Yamaguchi, N. H. (2019). Smoking, immunity, and DNA damage. In *Translational Lung Cancer Research* (Vol. 8). https://doi.org/10.21037/tlcr.2019.03.02

Zikriah. (2014). Uji Imunomodulator Ekstrak Etanol Jintan Hitam (Nigella Sativa L.) Terhadap Jumlah Total Leukosit, Persentase Limfosit, Persentase Monosit dan Kadar Interleukin-1β Pada Mencit Balb/C. In *Skripsi* (p. UIN Syarif Hidayatullah Jakarta).

Zuo, L., He, F., Sergakis, G. G., Koozehchian, M. S., Stimpfl, J. N., Rong, Y., Diaz, P. T., & Best, T. M. (2014). Interrelated role of cigarette smoking, oxidative stress, and immune response in COPD and corresponding treatments. In *American Journal of Physiology - Lung Cellular and Molecular Physiology* (Vol. 307, Issue 3). https://doi.org/10.1152/ajplung.00330.2013